Industrial Health 2013, **51**, 319–325

Original Article

The Increases in mRNA Expressions of Inflammatory Cytokines by Adding Cleaning Solvent or Tetrachloroethylene in the Murine Macrophage Cell Line J774.1 Evaluated by Real-time PCR

Takamasa KIDO[1, 2], Chiemi SUGAYA[1], Ryutaro IKEUCHI[1], Yuichiro KUDO[1], Masashi TSUNODA[1]* and Yoshiharu AIZAWA[3]

[1] Department of Preventive Medicine, Kitasato University School of Medicine, Japan
[2] Department of Public Health and Environmental Medicine, The Jikei University School of Medicine, Japan
[3] Kitasato University, Japan

Received December 20, 2011 and accepted February 13, 2013
Published online in J-STAGE March 29, 2013

Abstract: The use of a petroleum-derived cleaning solvent for dry cleaning, instead of tetrachloroethylene (perchloroethylene, PCE), has increased. The cleaning solvent may induce immunological alteration. In this study, murine macrophage-lineage J774.1 cells were exposed to the cleaning solvent at 0, 25, 50, and 75 μg/ml or PCE at 0, 400, 600, 800, and 1,000 μg/ml by vigorous vortexing. Cell viability was determined. The mRNA expressions of tumor necrosis factor-alpha (TNF-*α*), interleukin-1 beta (IL-1*β*), IL-6, IL-10, IL-12p40 (a dimer of IL-12), and IL-27p28 (a dimer of IL-27) were evaluated by real-time PCR. The mean viabilities in the 50 and 75 μg/ml groups of the cleaning solvent were significantly lower than that of the control. The mean mRNA expressions of TNF-*α* and IL-1*β* in the 50 μg/ml group were significantly higher than those in the control. For PCE, the mean viabilities at 600 μg/ml and over were significantly lower than that of the control. The mean expressions of IL-6 and IL-10 in the 800 μg/ml group were significantly higher than that in the control. The productions of IL-1*β* and TNF-*α* may be altered in human during intoxication of the cleaning solvent as well as those of IL-6 and IL-10 in human during that of PCE, and these may affect on immune cells.

Key words: Cleaning solvent, Tetrachloroethylene (Perchloroethylene, PCE), Macrophage, Cytokine, Real-time PCR

Introduction

The use of a petroleum-derived cleaning solvent for dry cleaning has increased recently. This type of cleaning solvent is currently used more than tetrachloroethylene (perchloroethylene, PCE) for cleaning, which has been reported for its toxic effects, such as hepatotoxicity, peripheral neuritis, chemical burn[1, 2]. The toxic effects of the cleaning solvent have been considered low, however, the solvent may cause allergic contact dermatitis[3]. Repeated exposure to the cleaning solvent would possibly induce allergy and inflammation among the workers at cleaning industries.

The roles of macrophages in inflammation and allergy

*To whom correspondence should be addressed.
E-mail: mtsunoda@med.kitasato-u.ac.jp

have been clarified[4]. Macrophages produce proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and IL-6[5]. IL-6 is also required for Th17 development which is related to allergic diseases such hypersensitivity penumonitis[6]. Macrophages produce IL-12, a heterodimer of p35 and p40 and which plays a role in allergy by inhibiting the development of the Th0/1 toward Th1 lymphocytes[7]. Some of the cytokines produced by macrophages have roles in anti-inflammation and allergy. IL-10 inhibits various inflammatory reactions and the production of cytokines by Th1 cells[8]. IL-10 is an anti-inflammatory cytokines, and induces a predominant Th2 reposnse[9]. IL-27, composed of EBI3 and p28, promotes the development of Th0 cells toward Th1 cells and inhibits immune reactions[10]. The productions of these cytokines by macrophages may be altered by the cleaning solvent or PCE.

J774.1 cells, a murine macrophage cell line, have been widely used as an *in vitro* model of macrophages[11–15]. The mRNA expressions of TNF-α, IL-1β, IL-6, IL-10, and IL-12p40 in J774.1 cells have been confirmed in our previous studies[11–15]. In addition, IL-27p28 may also be expressed in J774.1 cells. We examined the levels at which the cleaning solvent or PCE altered the mRNA expression of these cytokines in J774.1 cells. This information may be useful for determination of no observed adverse effect level (NOAEL) for these compounds if in vitro results are extrapolated in the actual exposure to these compounds.

In the laboratory, exposures of the cleaning solvent or PCE to the J774.1 cells are problematic. These organic materials do not mix well with the cell culture medium. Especially since the specific gravity of PCE is high, it sinks rapidly in the medium, until it reaches the bottom of the polystyrene plate, and finally dissolves it. Therefore, it was necessary to modify the methods for these exposures. Vigorous vortexing may be useful for the mixtures, and glass containers would not be dissolved by PCE. The cell viability and mRNA expressions of cytokines related to inflammation and allergy in J774.1 cells exposed to the cleaning solvent or PCE by vortexing were used as indexes.

The objective of this study was to elucidate alterations in the mRNA expression of cytokines in macrophages by the petroleum-derived cleaning solvent or PCE by using J774.1 cells.

Materials and Methods

Cell line

The murine macrophage cell line J774.1 was provided by the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai). J774.1 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium with penicillin G at 100 U/ml and streptomycin at 100 µg/ml (Nikken, Kyoto) with 5% [v/v] heat-inactivated fetal bovine serum (Equitec-Bio, Kerrville, TX) [11]. The viabilities of the cells were determined using trypan blue exclusion tests prior to the experiment. Cell populations with ≥90% viability were used in this study.

Exposure of J774.1 cells to cleaning solvent and PCE

For the exposure of the J774.1 cells to the cleaning solvent, 15 ml polystyrene tubes and a polystyrene, 24-well, cell culture plate (Iwaki, Tokyo, Japan) were used. For the exposure to PCE, 1.8 cm diameter, flat-bottom, glass weighing bottles (Sansho, Tokyo, Japan), precoated with 0.1% gelatin (w/v) solution, were used. The cleaning solvent and PCE was dissolved in ethanol and diluted in the culture medium. The J774.1 cells were exposed to the cleaning solvent at 0, 25, 50, and 75 µg/ml in the polystyrene tubes or exposed to PCE at 0, 400, 600, 800, and 1,000 µg/ml in the glass weighing bottles (n=6 per group). The final concentration of ethanol in each tube or bottle was 0.1%. The polystyrene tubes and the glass bottles were vigorously vortexed at least 30 s for mixing. The cells exposed to the cleaning solvent were disseminated to the wells of a 24-well plate. A total of 2×10^6 J774.1 cells was placed on 2.0 ml of RPMI 1640 medium in each well or bottle.

The plates or the glass bottles were incubated in a humidified incubator at 37 °C with 5% CO_2 for 6 h. After 6 h of incubation, lipopolysaccharide at a concentration of 100 ng/ml was added to each well and glass bottle. The cells were then incubated for an additional 6 h for analyses of mRNA expressions of cytokines followed by the previous studies[11, 13, 14]. The cell viability of each well and glass bottle was determined by the trypan blue exclusion test.

Real-time PCR

Total RNA was extracted from the J774.1 cells exposed to cleaning solvent or PCE after an additional 6-h incubation using RNeasy Mini Kit (QIAGEN, Tokyo, Japan). The cDNA was synthesized from mRNA using a First-Strand cDNA Synthesis Kit (GE Healthcare, Tokyo, Japan). Real-

time PCR was performed for TNF-α, IL-1β, IL-6, IL-10, IL-12p40, IL-27p28, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping gene using the Light Cycler System 2.0 (Roche Diagnostics, Tokyo, Japan). SYBER Green I was used for real-time PCR. The sense and anti-sense primers used were 5′-AACCTGCCAARTATGATGAC-3′ and 5′-TCATACCAGGAAATGAGCTT-3′ (R is a mixture of A and G) for GAPDH, 5′-ACAAGTGRTATTCTCCATGAGC-3′ (R is a mixture of A and G) and 5′-CCACTTTGSTCTTGACTTCTAT-3′ (S is a mixture of C and G), 5′-GTTCTCTGGGAAATCGTGGA-3′ and 5′-GGAAATTGGGGTAGGAAGGA-3′ for IL-6, 5′-CTCTTCAAGGGACAAGGCTG-3′ and 5′-CGGACTCCGCAAAGTCTAAG-3′ for TNFα, 5′-GACCAGCTGGACAACATACT-3′ and 5′-GAGGGTCTTCAGCTTCTCWC-3′ (W is a mixture of G and A) for IL-10, 5′-CTCTGTCTGCAGAGAAGGTC-3′ and 5′-GCTGGTGCTGTAGTTCTCAT-3′ for IL-12p40, 5′-CTCTGCTTCCTCGCTACCAC-3′ and 5′-GGGGCAGCTTCTTTTCTTCT-3′ for IL-27p28[16]. The thermal cycles consisted of denaturation at 94 °C annealing at 55 °C for GAPDH, IL-1β and IL-12p40, 54 °C for TNF-α, 52 °C for IL-6, 50 °C for IL-27p28 and extension at 72 °C. The calibrator normalized relative ratio for each cytokine to GAPDH was calculated as the index of mRNA expression of each cytokine followed by the method and formula previously described[13, 14].

Statistical analyses

The mean values of cell viabilities, the calibrator normalized relative ratios for cytokines in the groups were calculated. The data of the cleaning solvent- or PCE-exposed groups and the respective controls were compared by ANOVA (one-way analysis of variance) followed by Fisher's PLSD (protected least significant difference) test or non-parametric Kruskal-Wallis test by using StatView ver 4.51 (SAS Institute, Cary, NC).

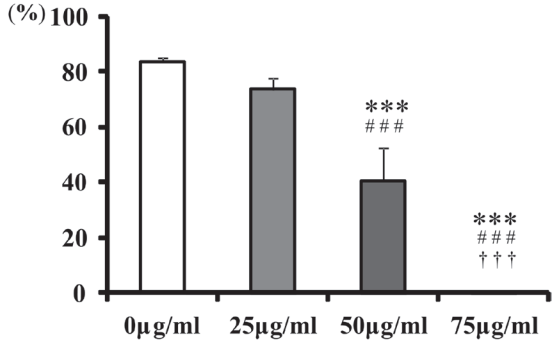

Fig. 1. Cell viabilities of J774.1 cells exposed to the cleaning solvent.

The J774. 1 cells exposed to the cleaning solvent in polyethylene tubes at the concentrations of 0, 25, 50 and 75 μg/ml and incubated in 24-well culture plates for 6 h. The cell viabilities were determined by trypan blue exclusion test. Each bar represents mean value, and each error bar represents standard error. p<0.0001 by ANOVA. ***p<0.001 (vs. control). ###p<0.001 (vs. 25 μg/ml). †††p<0.001 (vs. 50 μg/ml).

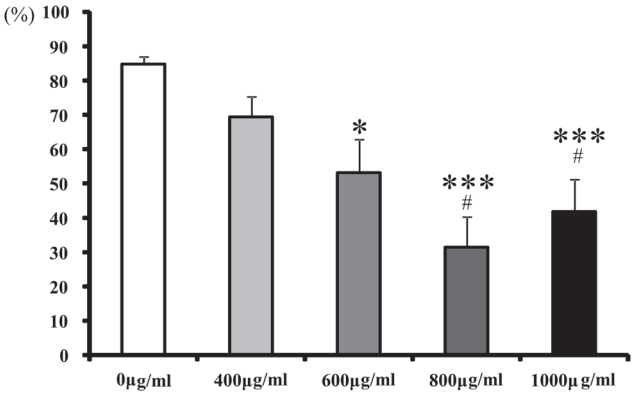

Fig. 2. Cell viabilities of J774.1 cells exposed to tetrachloroethylene (PCE).

The J774. 1 cells exposed to PCE in glass bottles at the concentrations of 0, 400, 600, 800 and 1,000 μg/ml and incubated in 24-well culture plates for 6 h. The cell viabilities were determined by trypan blue exclusion test. Each bar represents mean value, and each error bar represents standard error. p=0.0005 by ANOVA. *p<0.05 (vs. control). ***p<0.001 (vs. control). #p<0.05 (vs. 400 μg/ml).

Results

Figure 1 illustrates the cell viabilities of the J774.1 cells exposed to the cleaning solvent at 6 h after the activation. The mean values of the cell viabilities in the 50 μg/ml and 75 μg/ml groups were significantly lower than those in the control and the 25 μg/ml group. Figure 2 illustrates the cell viabilities of the J774.1 cells exposed to PCE at 6 h after the activation. The mean values of the cell viabilities in the groups exposed to ≥600 μg/ml were significantly lower than that in the control. The mean values of the cell viabilities in the groups exposed to ≥800 μg/ml were also significantly lower than that in the cells exposed to 400 μg/ml. For the mRNA expressions of cytokines in the J774.1 cells exposed to the cleaning solvent, the mean values of calibration normalized relative ratios are shown for TNF-α in Fig. 3 (a) and IL-1β in Fig. 3 (b). That for TNF-α in the 50 μg/ml group was significantly higher than those in the control and the 25 μg/ml group. That for IL-1β in the 50 μg/ml group was significantly higher than those in the control, 25 μg/ml, and 75 μg/ml groups. The mean values

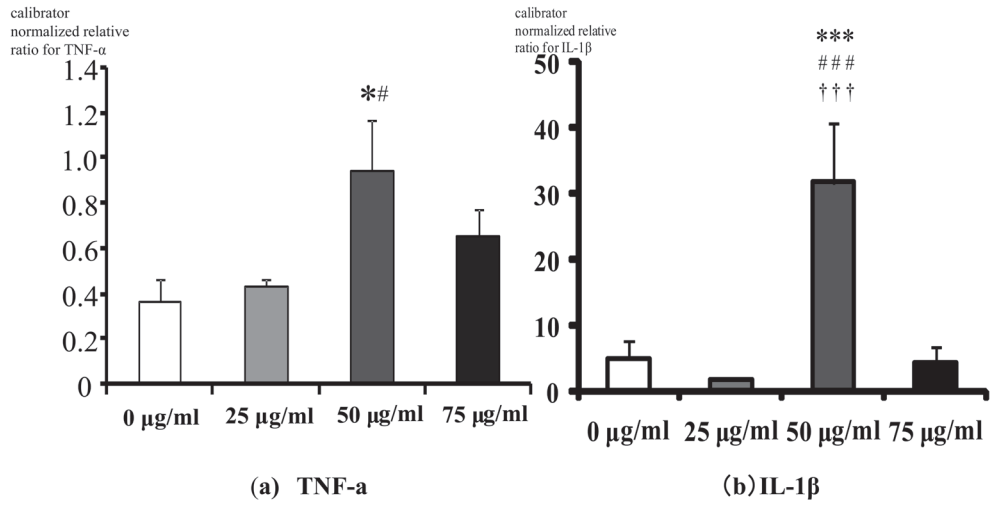

Fig. 3. The mRNA expression of TNF-α (a) and IL-1β (b) in the J774.1 cells exposed to the cleaning solvent.
The RNA was extracted from the J774. 1 cells exposed to the cleaning solvent for 6 hours. The cDNA was synthesized from mRNA and the real-time PCR was performed for TNF-α, IL-1β and GAPDH as a house keeping gene. The calibrator normalized relative ratio for TNF-α or IL-1β to GAPDH was calculated. Each bar represents mean value, and each error bar represents standard error. p=0.0301 by ANOVA for TNF-α and p=0.0007 by ANOVA for IL-1β. *p<0.05, ***p<0.001 (vs. control). #p<0.05, ###p<0.001 (vs. 25 μg/ml). †††p<0.001 (vs. 75 μg/ml).

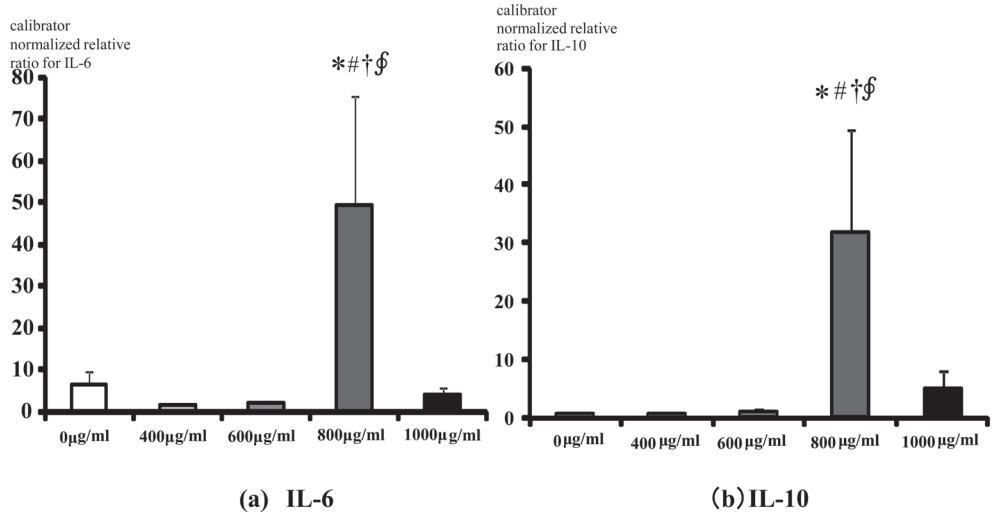

Fig. 4. The mRNA expression of IL-6 (a) and IL-10 (b) in the J774.1 cells exposed to tetrachloroethylene (PCE).
The RNA was extracted from the J774. 1 cells exposed to the cleaning solvent for 6 hours. The cDNA was synthesized from mRNA and the real-time PCR was performed for IL-6, IL-10 and GAPDH as a house keeping gene. The calibrator normalized relative ratio for IL-6 or IL-10 to GAPDH was calculated. Each bar represents mean value, and each error bar represents standard error. p=0.0344 by ANOVA for IL-6 and p=0.0455 by ANOVA for IL-10. *p<0.05 (vs. control). # p<0.05 (vs. 400 μg/ml). †p<0.05 (vs. 800 μg/ml). ʃp<0.05 (vs. 1,000 μg/ml).

of the calibration normalized relative ratios for IL-10 and IL-12p40 in the J774.1 cells exposed to the cleaning solvent are shown in Table 1. Because the values were not normally distributed, the non-parametric Kruskal-Wallis test was applied for the comparisons among the groups. There were significant differences among the IL-10 and IL-12p40 groups. The medians of IL-10 and IL-12p40 in the 75 μg/ml groups were high. There were no significant differences among the groups exposed to the cleaning solvent for the calibration normalized relative ratios for IL-6

Table 1. Expressions of mRNA of IL-10 and IL-12p40 in the J774.1 cells exposed to cleaning solvent

	Calibrator normalized relative ratio for IL-10		*p*-value
0 μg/ml	0.349 ± 0.127[a]	0.306[b]	0.0098[c]
25 μg/ml	0.145 ± 0.077	0.075	
50 μg/ml	4.024 ± 2.699	0.832	
75 μg/ml	1.637 ± 0.634	1.114	
	Calibrator normalized relative ratio for IL-12p40		
0 μg/ml	4.890 ± 2.460	2.297	0.0033
25 μg/ml	0.285 ± 0.130	0.163	
50 μg/ml	13.131 ± 6.111	6.880	
75 μg/ml	11.600 ± 3.209	14.694	

[a]Mean ± SE, [b]Median, [c]Kruskal-Wallis test.

Table 2. Expressions of mRNA of IL-6 and IL-27p28 in the J774.1 cells exposed to cleaning solvent

	Calibrator normalized relative ratio for IL-6		*p*-value
0 μg/ml	2.245 ± 1.145[a]	1.189[b]	0.473[c]
25 μg/ml	0.754 ± 0.157	0.732	
50 μg/ml	1.136 ± 0.410	1.102	
75 μg/ml	1.537 ± 0.603	1.070	
	Calibrator normalized relative ratio for IL-27p28		
0 μg/ml	0.525 ± 0.122	0.449	0.2419
25 μg/ml	0.557 ± 0.121	0.458	
50 μg/ml	1.761 ± 0.796	1.118	
75 μg/ml	1.457 ± 0.686	0.615	

[a]Mean ± SE, [b]Median, [c]Kruskal-Wallis test.

and IL-27p28 (Table 2).

The mean values of calibration normalized relative rations in the J774.1 cells exposed to PCE are shown for IL-6 in Fig. 4 (a) and IL-10 in Fig. 4 (b). That for IL-6 in the 800 μg/ml group was significantly higher than those in the 0, 400, 600, and 1,000 μg/ml groups. That for IL-10 in the 800 μg/ml group was also significantly higher than those in the 0, 400, 600, and 1,000 μg/ml groups. These were no significant differences among the groups exposed to PCE for the calibration normalized relative ratios for TNF-α, IL-1β, IL-12p40, and IL-27p28 (Table 3).

Table 3. The mRNA expressions of cytokines in the J774.1 cells exposed to tetrachloroethylene (PCE)

	Calibrator normalized relative ratio for TNF-α		*p*-value
0 μg/ml	3.944 ± 0.518[a]	3.908[b]	0.5131[c]
400 μg/ml	3.056 ± 0.364	2.971	
600 μg/ml	2.439 ± 0.182	2.336	
800 μg/ml	2.699 ± 1.622	1.148	
1000 μg/ml	21.004 ± 19.581	2.229	
	Calibrator normalized relative ratio for IL-12p40		
0 μg/ml	5.528 ± 1.647	5.500	0.1317
400 μg/ml	2.767 ± 1.105	1.531	
600 μg/ml	5.042 ± 2.253	3.110	
800 μg/ml	16.538 ± 8.051	7.915	
1000 μg/ml	4.817 ± 1.574	3.100	
	Calibrator normalized relative ratio for IL-1β		
0 μg/ml	7.112 ± 2.090	5.596	0.7207
400 μg/ml	3.658 ± 0.525	3.767	
600 μg/ml	5.492 ± 1.990	2.726	
800 μg/ml	38.327 ± 23.229	6.592	
1000 μg/ml	9.750 ± 5.805	3.936	
	Calibrator normalized relative ratio for IL-27p28		
0 μg/ml	0.680 ± 0.165	0.514	0.8665
400 μg/ml	0.463 ± 0.049	0.437	
600 μg/ml	0.621 ± 0.112	0.564	
800 μg/ml	31.459 ± 26.578	3.429	
1000 μg/ml	8.678 ± 5.900	0.656	

[a]Mean ± SE, [b]Median, [c]Kruskal-Wallis test.

Discussion

The various toxic effects of PCE have been reported in industries[1]. It has been known as a primary irritant and induces inflammation in the liver[2, 17]. Therefore, because of low toxicities of the petroleum-derived cleaning solvent, the use of the cleaning solvent has increased in the cleaning industry. However, Hanai *et al*. (2005) suggested that the cleaning solvent causes irritant contact dermatitis. It is of interest to know which mechanisms work on inflammation induced by the cleaning solvent or PCE. To elucidate the toxicity mechanisms of the cleaning solvent and PCE, it is useful to use an *in vitro* model. J774.1 cells, a murine macrophage lineage, are cultivated relatively easily, and produce various cytokines related to allergy and inflammation. The cell line has been used as an *in vitro* model for macrophages in previous studies[11–15]. We confirmed the mRNA expression of IL-27p28, which inhibits the production of inflammatory cytokines by various cells[10], in the J774.1 cells in this study. Therefore, we used J774.1 cells in this study, and IL-27p28 mRNA expression in J774.1 cells was used as one of the indexes for toxicity in addition to other cytokines used in our previous studies[13, 14].

PCE, which has a high gravity, sinks in the media and, when it reaches the bottom of the polystyrene cell culture plates, it dissolves it. The dissolved material derived from the plates was toxic to the cells. We solved this problem by using glass bottles coated with gelatin instead of the

polystyrene cell culture plates. In the bottles, J774.1 cells adhered to the bottom and were cultivated. The exposure to PCE became possible in the glass bottles. Both the cleaning solvent and PCE are organic materials and are difficult to mix with culture medium. We tried to overcome this difficulty of mixing these organic solvents with the cell medium by vortexing them vigorously. We could observe cell death in high concentrations of these materials. Vortexing was useful for mixing to a certain extent.

Regarding the cleaning solvent, the mean values of cell viabilities in the groups exposed to 50 μg/ml and over were significantly lower than that in the control. The cell death induced at 50 μg/ml could indicate one of the mechanisms that induces irritant contact dermatitis. The mean values of mRNA expressions of TNFα and IL-1β in the groups exposed to 50 μg/ml were significantly higher than those in the control and 25 μg/ml groups. Because cell death occurs at the level of 50 μg/ml, the cells may express more mRNAs of TNFα and IL-1β during the process of cell death. The cleaning solvent may induce dermatitis by the increase of the production of inflammatory cytokines at 50 μg/ml. Regarding IL-10 and IL-12p40, which are anti-inflammatory cytokines[3], calibrator-normalized relative rations at 75 μg/ml were higher than those in the control. Although the J774.1 cells may express more IL-10 and IL-12 at this high level, it is noteworthy that the most J774.1 cells were killed. The release of IL-10 and IL-12 from J774.1 cells may not be increased over all. There were no significant differences among the groups regarding IL-27p28, suggesting that this cytokine is not related to the toxicity of the cleaning solvent.

Regarding PCE, the mean values of the cell viabilities in the groups at 600 μg/ml and over were significantly lower than that in the control. Because the gravity of PCE is high, this level should be carefully compared to the level that induced cell death by the cleaning solvent. PCE may be less soluble in the media than the cleaning solvent. Therefore, the concentration that induced cell death may be higher for PCE. Regarding the mRNA expressions of cytokines, only the group exposed to 800 μg/ml showed increases in IL-6 and IL-10 compared with that in the control. However, there were no significant differences among the groups in the expressions of proinflammatory cytokines. IL-6 is a multipotent cytokine that is related to various reactions in addition to inflammation[4]. The toxic effects of PCE such as hepatotoxicity may be mediated by IL-6, which is closely related to hepatotoxicity induced by ethanol[17]. The significant increase in mRNA expression of IL-10 at 800 μg/ml may be compensatory for the increased mRNA expression of IL-6.

There were also no significant differences among the groups exposed to PCE for the mRNA expression of IL-27p28, suggesting no relation to the toxicity of PCE.

Whether or not the decrease in the number of living cells and the increase in the mRNA expressions of the cytokines results in the increase of release of cytokines from the cells remains to be determined. Determinations of the cytokine proteins in the supernatant from the J774.1 cells by enzyme-linked immunosorbent assay (ELISA) are needed. However, the mixed solvents media and organic solvents may have interfered with the reactions of ELISA. Separation of the organic solvents from the media may be required to determine the proteins in the media by ELISA.

PCE has been known as an environmental pollutant in groundwater and soil[18, 19]. The carcinogenecity of PCE was demonstrated in experimented animals[20]. From these facts, the use of the cleaning solvent has been recommended as an alternative for PCE as a dry cleaning solvent. However, in this study, although it should be careful to compare the levels that induced macrophage death and inflammation between the cleaning solvent and PCE, the level that of the cleaning solvent was 50 μg/ml, which was much lower than that of PCE. Although it is still recommended that the cleaning solvent be used as a dry cleaning solvent in spite of inflammatory effect, to prevent contact dermatitis, the use of the gloves are strongly recommended for workers engaged in the dry cleaning process. It is also necessary to be careful regarding the residue on the clothes processed using the cleaning solvent for dry cleaning workers and customers.

The mRNA expressions of various cytokines including IL-27p28 in J774.1 cells were confirmed in this study, which may be useful for the evaluation of other toxic materials. In further studies, evaluation using epidemic cells or T lymphocytes may be useful for elucidation of the mechanism of irritant contact dermatitis related to cleaning solvent.

In this study, the increases in cell death and mRNA expressions of inflammatory cytokines detected by real-time PCR in the J774.1 cells induced by the cleaning solvent were observed. These effects may be related to the onset irritant contact dermatitis induced by the cleaning solvent. For PCE, the increase in the mRNA expression of IL-6 was induced, which may be related to hepatotoxicity.

References

1) Lash LH, Parker JC (2001) Hepatic and renal toxicities

associated with perchloroethylene. Pharmacol Rev **53**, 177–208.
2) Miyano N, Nakano Y (1987) Effect of trichloroethylene, perchloroethylene and 1,1,1-trichloroethane on immune responses. Proc Osaka Prefectural Inst Public Health Edition Ind Health **25**, 15–9 (in Japanese).
3) Hanai H, Terui T, Suzuki H (2005) Clinical example of irritant contact dermatitis. Prac Dermatol **27**, 1027–30 (in Japanese).
4) Yada J (2005) Textbook of immunology for medical students and physicians, 9th Ed., Chugai-Igakusha Tokyo (in Japanese).
5) Yoshimura A, Hasegawa E, Muto E (2010) Inflammation and cytokines. Exp Med **28**, 1828–36 (in Japanese).
6) Abdelsamed HA, Desai M, Nance SC, Fitzpatrick EA (2011) T-bet controls severity of hypersensitivity pneumonitis. J Inflamm (Lond) **8**, 15.
7) Takeuchi O (2010) Regulatory mechanisms of cytokine production in macrophages and dendritic cells. Exp Med **28**, 1837–41 (in Japanese).
8) Morishima N, Chiba Y, Yoshimoto T (2010) Anti-inflammatory cytokines IL-10 and IL-35. Exp Med **28**, 1916–22 (in Japanese).
9) Deo SS, Mistry KJ, Kakade AM, Niphadkar PV (2010) Role played Th2 type cytokines in IgE mediated allergy and asthma. Lung India **27**, 66–71.
10) Yoshida H (2010) Regulation of immune responses and inflammation by IL-27. Exp Med **28**, 1954–9 (in Japanese).
11) Hosokawa M, Inoue Y, Chen XF, Tsunoda H, Ito K, Katagiri H, Tsunoda M, Aizawa Y (2011) The alterations in cytokine mRNA expressions and productions by fluoride in a murine macrophage cell line evaluated by real-time PCR and ELISA. Biomed Res Trace Elem **22**, 27–33.
12) Hosokawa M, Sugaya C, Inoue Y, Tsunoda M, Aizawa Y (2009) Cytoxity and inhibition of the production of tumor necrosis factor α and interleukin-1β induced macrophage cell line. Fluoride **42**, 188–97.
13) Tsunoda M, Yoshida T, Tsuji M, Zhang Y, Sugaya C, Inoue Y, Miki T, Kudo Y, Satoh T, Aizawa Y (2008) The effects of dibutyltin (DBT) dichloride on the viability and the productions of tumor necrosis factor α and interleukin-12 in murine macrophage cell line J774.1. Biomed Res Trace Elem **19**, 67–71.
14) Tsunoda M, Yamamoto K, Ito K, Inoue Y, Miki T, Kudo Y, Satoh T, Aizawa Y (2006) dibutyltin (DBT) dichloride inhibits cytokine productions in murine macrophage cell line J774.1. Biomed Res Trace Elem **17**, 417–22.
15) Nakano K, Tsunoda M, Konno N (2004) Tributyltin (TBT) increases TNFα mRNA expression and induces apoptosis in the murine macrophage cell line in vitro. Environ Health Prev Med **9**, 266–71.
16) Liu J, Guan X, Ma X (2007) Regulation of IL-27p28 gene expression in macrophages through MyD88-and interferon-γ-mediated pathways. J Exp Med **204**, 141–52.
17) Naveau S, Cassard-Doulcier AM, Njiké-Nakseu M, Bouchet-Delbos L, Barri-Ova N, Boujedidi H, Dauvois B, Balian A, Maitre S, Prévot S, Dagher I, Agostini H, Grangeot-Keros L, Emilie D, Perlemuter G (2010) Harmful effect of adipose tissue on liver lesions in patients with alcoholic liver disease. J Hepatol **52**, 895–902.
18) Jawitz JW, Sillan RK, Annable MD, Rao PSC, Warner K (2000) In-situ flushing of a DNAPL source zone at a dry cleaner site. Environ Sci Technol **34**, 3722–9.
19) Hoggan JL, Bae K, Kibbey TC (2007) Non-steady partitioning of dry cleaning surfactants between tetrachloroethylene (TCE) and water in porous media. J Contam Hydrol **93**, 149–60.
20) NTP (1986) Toxicology and carcingenesis studies of tetrachloroethylene (perchloroethylene) (CAS No. 127–18-4) in F344/N rats and B6C3F1 mice (Inhalation Studies). Natl Toxicol Program Tech Rep Ser. No 311.